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PRINCIPAL INVESTIGATOR: Lynn C. Hartmann, M.D.

CONTRACTING ORGANIZATION: Mayo Clinic
Rochester, MN 55905

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Abstract

Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. Unfortunately only a minority of the over 200,000 women who are diagnosed with breast cancer in the US each year are recognized as being at significantly increased risk. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical arena. This will require progress on three fronts of scientific investigation: (i) establishment of a tissue repository of benign breast disease (BBD); (ii) assessment of potential biomarkers of risk in this tissue set and (iii) discovery of new, potentially relevant biomarkers of risk. We have made significant progress on these aims. Our 25-year cohort includes 9,087 women with BBD, 707 of whom have developed a breast cancer to date. We have completed the cohort follow-up by questionnaire. All benign histopathology has been read. For those women who developed breast cancer, we have cancer tissue for 93% of those diagnosed at Mayo and 55% diagnosed outside Mayo. We are studying biomarkers (COX-2, centrosome status, cyclin D1, etc) in several relevant subsets including atypia. For discovery, 14 BBD samples are growing successfully thus far.

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INTRODUCTION

This is our third year Center of Excellence report; however, it details a total of only 28 months of work involving human subjects because of delays in the start-up funding of that portion of the grant. The purpose of our Center of Excellence is to bring molecular risk prediction for breast cancer into the clinical arena. There are three main areas of scientific activity within this Center: 1) the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-1991) with complete and long-term clinical follow-up to identify those who developed breast cancer (cases) and those who did not (controls); 2) the application of potential biomarkers of risk to this archival tissue set; and, 3) the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD. The Center includes a multi-institutional team of basic scientists, pathologists, epidemiologists, clinicians, statistician, and advocates (Mayo Clinic; University of California San Francisco (UCSF); Wayne State).

I. Task 1: Establish Retrospective Cohort of BBD and Nested Case-Control Study

A. Complete cohort follow-up

We have now combined what was previously referred to as cohort I (1967-81 group) and cohort II (1982-91 group). The overall 25-year cohort inclues 9,087 women, followed a median of 15 years. Seven hundred and seven women have been diagnosed with breast cancer. The median time from breast biopsy to the diagnosis of breast cancer is 10.7 years.

B. Validate reported breast cancers

Charts and questionnaire data were reviewed to validate all breast cancers. Four hundred ten (58%) of the women with breast cancer were diagnosed at Mayo Clinic and 297 (42%) were diagnosed outside of Mayo Clinic. For women diagnosed outside of Mayo Clinic, a contact was initiated to obtain permission to access medical records associated with their breast cancer diagnosis and their breast cancer tissue. To date we have received breast cancer blocks on 381 (93%) of women diagnosed at Mayo Clinic and 163 (55%) of the women diagnosed outside of Mayo Clinic. We have slides on an additional 3% of the women diagnosed at Mayo Clinic and 7% of the women diagnosed outside of Mayo Clinic. Work remains in process to collect as much of the breast cancer tissue as possible.

C. Match appropriate controls to known breast cancer cases

We described our matching procedure in last year's report. This task has been completed.

D. Construct test set for preliminary evaluation of markers

We described the construction of our test set in last year's report. This subset is comprised of 124 cases and their two closest controls selected from the entire study period.

E. Construct validation set from remaining breast cancer cases, each matched with two controls

The remaining cases and controls will serve as the validation set.

II. Task 2: Biomarkers in Archived Tissues from Cases and Controls

A. Retrieve tissue slides/blocks of BBD specimens for all cases and controls

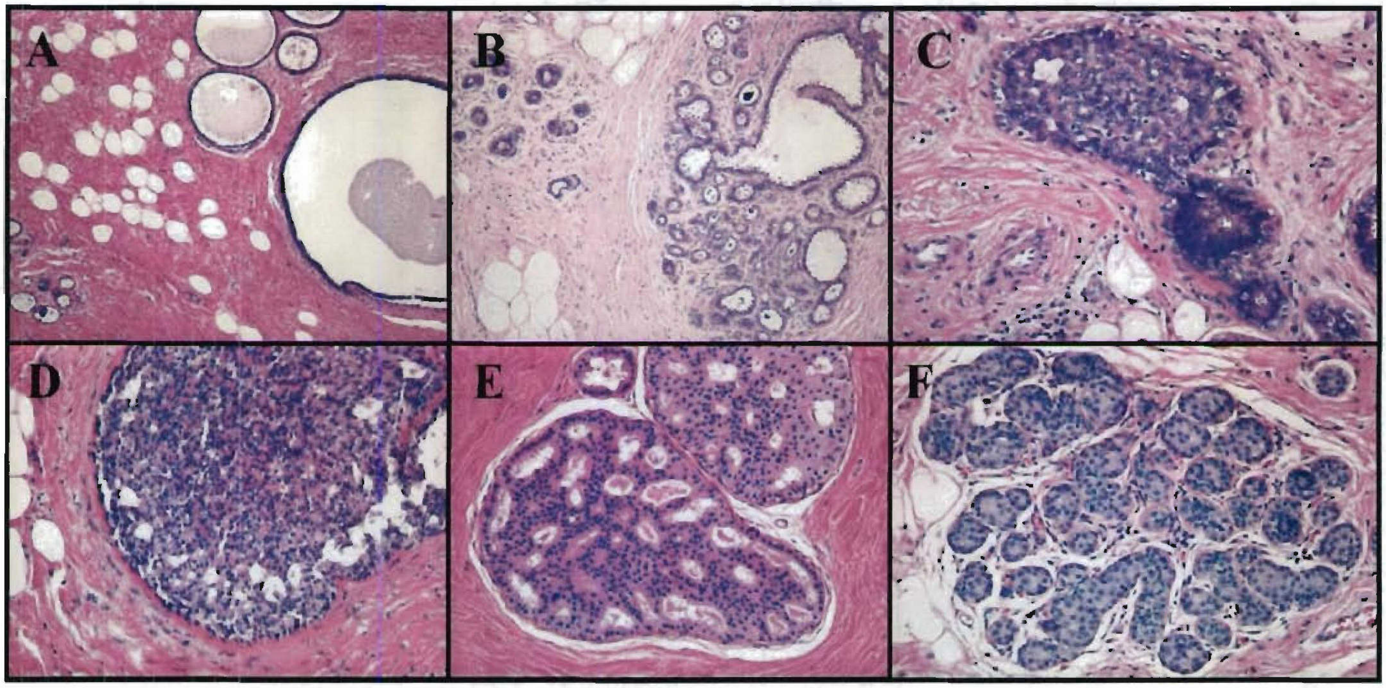
Archived paraffin blocks and slides for the 9,087 women were obtained from the Mayo Clinic Tissue Registry. For tracking purposes, the pathology numbers, assigned at biopsy to the blocks and corresponding slides, are entered on SAS screens in the data set for each patient. Our tracking system in our database identifies the location of those blocks and slides at any point in time.

B. Characterize benign histopathology

Dr. Dan Visscher (breast pathologist), blinded to the initial histologic diagnoses and patient outcomes, has characterized all the benign histopathology according to the criteria of Page et al[1-2] into the following categories: non-proliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes with atypia (atypical ductal hyperplasia and/or atypical lobular hyperplasia). Biopsies were designated as having proliferative fibrocystic change if they contained any of the following: duct hyperplasia (greater than mild), papilloma, radial scar or sclerosing adenosis. Representative examples are included in the accompanying figure. Cysts, fibroadenoma or columnar changes were considered non-proliferative unless they also contained one of the lesions denoted above.

The broad histologic classifications included: non-proliferative disease, 6,061 (66.7%); proliferative disease without atypia, 2,690 (29.6%); and atypical hyperplasia, 336 (3.7%).

Histopathology of Benign Breast Disease



A. Non-proliferative: Terminal duct lobular unit architecture is distorted by the formation of microcysts, associated with interlobular fibrosis (150X).
B. Proliferative without atypia – adenosis: A distinctive form of hyperplasia characterized by proliferation of lobular acini, forming crowded gland-like structures (150X). For comparison, a normal lobule is seen on the left.
C. Proliferative without atypia – ductal hyperplasia, moderate: A duct that is partially distended by hyperplastic epithelium within the lumen characterizes moderate duct hyperplasia (250X).
D. Proliferative without atypia – ductal hyperplasia, florid: The involved duct is significantly expanded by a crowded, jumbled appearing, epithelial proliferation (250X).
E. Atypical ductal hyperplasia: These proliferations are characterized by a combination of architectural complexity, with partially formed secondary lumens, and mild nuclear hyperchromasia in the epithelial cell population (250X).
F. Atypical lobular hyperplasia: Monotonous cells fill the lumens of partially distended acini in this terminal duct lobular unit (250X).

C. Prepare tissue slides for biomarker analyses

Tissue slides have been prepared for the test set and two other subgroups of interest including those for women whose breast cancer occurred within 5 years of their diagnosis (n = 174) and women whose histopathology revealed atypia (n = 336).

D. Perform IHC for MIB-1, ER, p53

These specific biomarker studies were put forward in our original application as those most likely to be performed. Which biomarkers to analyze in these precious and limited samples of benign breast disease is the number one topic of discussion among our Center investigators, and with our advocate advisors. Our focus continues to be on the earliest possible changes that we might detect in these "premalignant" lesions. During the June 2005 DOD Era of Hope meeting, there was much discussion on the identification of premalignant lesions and possible biomarkers to study in them. There is certainly no consensus on this point. With input from Dr. Tlsty at University of California, San Fransisco (our senior scientist on the Center grant) we have proceeded to stain all our atypia samples with cox-2 by IHC.[3] These samples are currently being read by our study pathologist. Representative samples are included in Appendix A.

The other IHC work that we have definitely committed to is that of Dr. Junjie Chen at Mayo Clinic, who recently received a DOD Era of Hope Scholar award. Dr. Chen's work focuses on the activation of DNA damage pathways, now thought to be one of the earliest detectable anomalies in premalignant lesions.[4-5]

E. Perform FISH

Regarding FISH for centromere and locus-specific probes, these markers were put forward in our original grant application as a way of looking for aneusomy (i.e. genomic instability) and to evaluate possible relevant loci. We have since determined that analysis of centromeres, described below, is an efficient marker of chromosomal instability that does not require as much tissue as FISH. The comments made above regarding the IHC studies pertain to the FISH studies. Every marker that we might evaluate consumes 5 microns from the paraffin block. We are approaching all the biomarkers with the same degree of caution since these benign breast biopsy samples are small and easily consumed. We have the techniques to do FISH probes worked out for the specimens. The question remains which biomarkers are most relevant to test. At this time, we are proceeding with the centrosome studies (see below) and cox-2 by IHC. We anticipate starting the DNA damage response pathway studies in the next half year. Whether or not we need to do FISH analyses for any of the biomarkers remains to be seen at this time.

F. Perform centromere studies

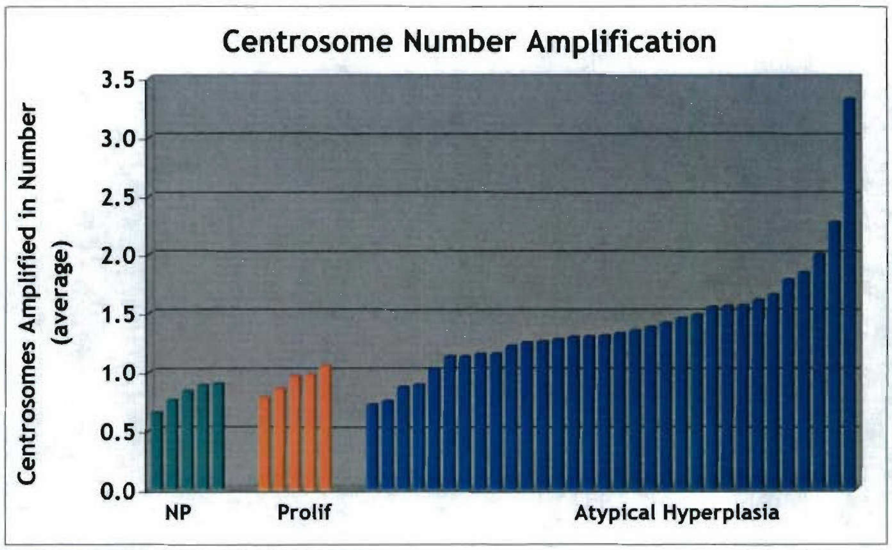
Most invasive breast cancers, like many other solid tumors, have amplified centrosomes. The extent of centrosome amplification correlates with the levels of chromosomal instability in invasive ductal carcinoma of the breast. Centrosome amplification is also present in ductal carcinoma in situ, but has not been investigated in benign breast lesions. In our pilot study we investigated the status of centrosomes in benign breast lesions of various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and if any histologic types of benign breast lesions have significant levels of centrosome amplification.

We selected paraffin-embedded tissue blocks from women with non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. We had previously determined the relative risks of developing breast cancer associated with these lesions in our large cohort of women; the relative risk associated with non-proliferative lesions was 1.27 (95% CI 1.15-1.41), 1.88 (95% CI 1.66-2.12) in proliferative lesions without atypia, and 4.24 (95% CI 3.26-5.41) in lesions with atypia. Serial sections were cut to allow for staining with hematoxylin and eosin, gamma tubulin, and cyclin D1 on adjacent slides. The lesions of interest were circled by the pathologist (DV) on the H&E or cyclin D1 stained slides. These slides were then scanned using a digital imaging system. The corresponding area was marked on the immunofluorescent slide stained with gamma tubulin antibodies to facilitate locating the lesion at high magnification.

Centrosome amplification was seen infrequently in non-proliferative lesions and in proliferative lesions without atypia. However, about 88% of atypical hyperplasia lesions had detectable centrosome amplification with about 30% having moderate to considerable levels of centrosome amplification (see Figures 1 and 2 below). Thus, centrosome amplification is seen more frequently in benign lesions having the highest relative risk of developing breast cancer.

This is the first quantitative demonstration of centrosome amplification in benign breast lesions. These pilot data demonstrate that centrosome amplification is more prevalent in the atypical hyperplasia lesions, and these lesions are associated with the highest relative risk of developing breast cancer.

(The centrosome work has been performed in Dr. Wilma Lingle's lab. She presented this work at the 2005 Annual Meeting of the American Association of Cancer Research.)



FFigure 2. Each bar represents the average centrosome number in an individual lesion. More than one centrosome per cell on average is found in 88% of atypical hyperplasia samples, compared to only 9% of the other BBD types. The range is also greater in atypical hyperplasias.

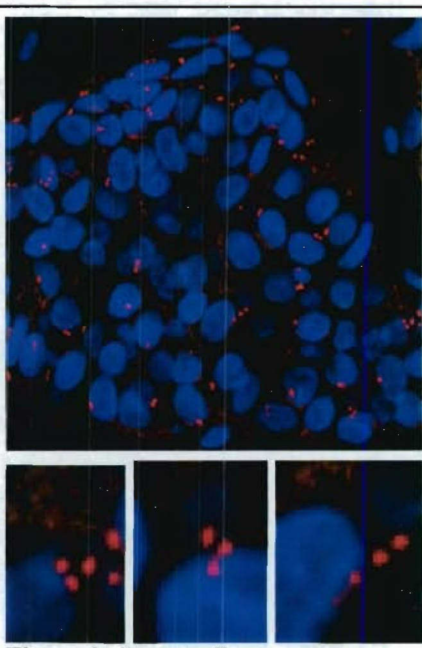


Figure 1. Immunofluorescence staining for centrosomes (red) in an atypical hyperplasia. Many nuclei (blue) have more than 2 centro-somes associated with them. Normal cells have 1 centrosome during G1 of the cell cycle and 2 centrosomes during G2 of the cell cycle.

III. Task 3: Discovery – *In Vitro* Culturing and Gene Profiling Studies

A. Identify appropriate frozen proliferative BBD specimens at Mayo and Wayne State for profiling

The purpose of these studies is to identify new, potentially relevant biomarkers in benign breast disease, markers that would correlate strongly with subsequent breast cancer risk. When our grant was submitted, the technology was not available to do profiling studies in paraffin-embedded tissue (such as our BBD resource) and hence, we described doing profiling in frozen samples of BBD. A serious limitation of that approach, however, is that we do not have outcome information for our frozen repository samples, since these were accrued recently, and insufficient time has elapsed for the development of breast cancer. Fortunately, genomic profiling technology has proceeded significantly and there now are platforms available for us where microdissected, paraffin-embedded samples can be run. We are working currently to identify the best platform for this purpose.

B. Obtain fresh BBD tissue from appropriate patients at Mayo and Wayne State for culturing in vitro at UCSF

To date, we have sent 44 samples to UCSF, of which only 5 were lost to contamination. Ten samples contained frozen digested material and UCSF will attempt to culture these samples again at a future time. UCSF currently has 14 samples growing. We continue to collect these tissues.

C. Culture a total of 80 BBD specimens and document their growth characteristics

The 14 samples growing provide the preliminary growth curves and characteristic micrographs shown in the figure below. Since all cultured samples have bypassed the first growth plateau, Dr. Tlsty's group is currently evaluating methylated p16 promoter status by PCR analysis of bisulfite modified DNA. Of these 14 cell culture samples, 8 were generated from the breast tissue of pre-menopausal women and 5 from post-menopausal breast tissue (one sample from a woman with unknown menopausal status). UCSF extracted RNA from these samples and reverse transcribed first and second strand cDNA using Ambion Message Amp kit. They are currently amplifying, biotinylating and in vitro transcribing the cDNA back into RNA for Affymetrix microarray analysis.

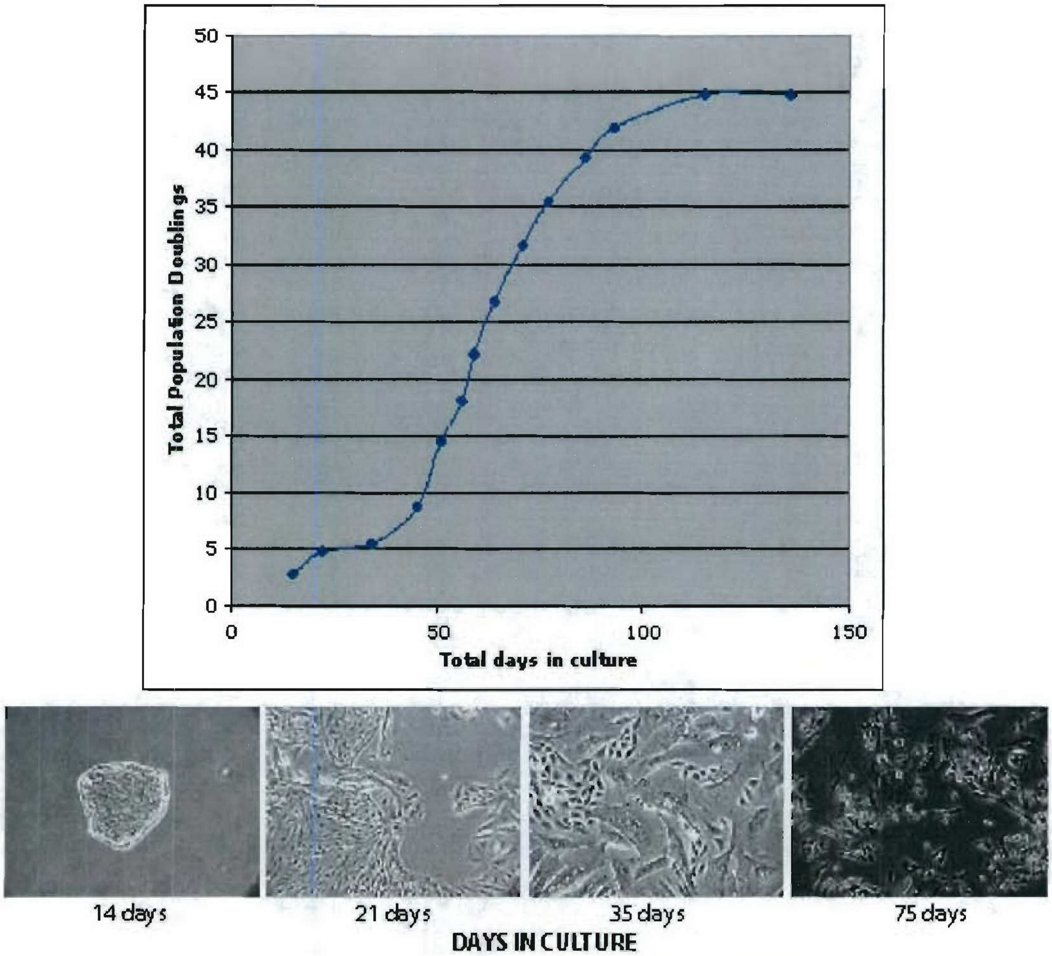


Figure 1. Representative growth curve and micrographs (4X) of BBD samples grown in culture.

D. <u>Compare genomic expression levels of DCIS markers in BBD tissues</u>

We described in our grant proposal using DCIS samples as a springboard for the identification of potentially relevant biomarkers in BBD. We have identified a cohort of 165 women who had DCIS diagnosed and treated at Mayo in the 1970s and early 1980s. We have obtained outcome information for these women, including the identification of those women who went on to have recurrent disease. We are in the process of creating a tissue microarray from these samples for marker testing. Markers that prove to be promising in the DCIS samples can then be moved into Task 2, to be tested in the BBD samples.

E. <u>Profile BBD specimens (proliferative versus normal; African American versus Caucasian; BBD with PROG versus no PROG; cultured BBD versus cultured normal)</u>

See discussion under III.A above. There now exists the technology to profile small samples from paraffin-embedded samples which allows us to pursue this task. We are working with Dr. Tlsty to identify the best platform for these studies.

IV. Task 4: Statistical Analyses

A. Establishment of relational database

In our 2003 report, we described the creation of our Sybase database. We continue to refine this database as we activate various components of the overall Center and the investigators further clarify the data they want to collect and report.

B. Enter epidemiologic and histopathologic data

We continue to enter data for the various aspects of our study. The following highlights those activities.

- All benign histology data read on the entire cohort have been entered.
- All benign slides have been entered on the entire cohort.
- Slides have been entered on all the obtained cancer tissue.
- We have documented to the extent possible the breast cancers. We verified the breast cancer by medical records and recorded the histopathology, tumor size, nodal status, metastasis, date of diagnosis, recurrence information when available, estrogen and progesterone status, and type of surgery. As new breast cancers are identified, they are also documented.
- All data from the questionnaires have been entered.
- We have begun the entry of cox-2 data.
- We have begun the entry of cancer histology.

We continue to do data clean-up on these sections as we prepare the data for use in manuscripts.

C. Enter culturing data (proportion of cells that break through proliferation barriers; slope of curve, etc.)

These data are being entered as collected at UCSF.

D. Enter molecular data from culturing experiments (methylation of p16, p53 status, % proliferation versus apoptosis, etc.)

These data are being entered as collected at UCSF.

E. Enter gene profiling data

See discussion under sections III.A and III.E. The technology is now available for us to pursue these experiments.

F. Calculate hazard function for breast cancer by age at BBD, family history, histology, and molecular marker data

We have examined breast cancer by age at BBD, family history, and histology. We are beginning to explore centrosome and Cox2 data.

We found that the histological classification of a benign breast lesion and a family history of breast cancer are risk factors for the development of breast cancer after the diagnosis of benign breast disease. To date, 707 breast cancers have developed in our cohort. The relative risk of breast cancer for the overall cohort is 1.56 (95% CI 1.45-1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95% CI 3.26-5.41), for proliferative changes without atypia it was 1.88 and for non-proliferative lesions it was 1.27 (95% CI 1.15-1.41). Family history was a risk factor independent of histology: for a strong family history, the relative risk was 1.93 (95% CI 1.58-2.32); for no family history it was 1.18 (95% CI 1.01-1.37). No increased risk was found among women with a negative family history and non-proliferative findings. In the first 10 years after the initial biopsy, an excess of cancers occurred in the same breast, especially in women with atypia, consistent with the presence of precursor lesions. These data are in press at the *New England Journal of Medicine.*

Over our 25-year cohort, the proportion of women with atypical hyperplasia increased from 1.3% in the first five years of the study to 6% in the last five years of the study. Similarly, the proportion of proliferative disease without atypia also increased from 24.1% in the early 70s to 34.8% in the late 1980s.

We identified 368 women with a single papilloma without atypia and 35 (10%) developed carcinoma. Eleven (22%) of the 49 women with a single papilloma with atypia subsequently developed carcinoma. Forty-one patients were diagnosed with multiple papillomas without atypia, and six (15%) developed carcinoma. Twelve cases of multiple papillomas without atypia were identified, and 4 (33%) of these developed carcinoma. See the relative risk table below. A manuscript further detailing these data has been submitted for publication.

Diagnosis (N)	Person Years Follow-up	Relative Risk* (95% CI)
Non-Proliferative	91129	1.00
Proliferative without Atypia	32895	1.60 (1.35,1.90)
Proliferative with Atypia	3127	3.59 (2.63,4.92)
Single Papilloma without Atypia	4979	1.82 (1.28, 2.58)
Single Papilloma with Atypia	577	4.88 (2.67, 8,92)
Multiple Papillomas without Atypia	592	2.81 (1.25, 6.31)
Multiple Papillomas with Atypia	115	8.66 (3.22, 23.31)

*Calculated using a Cox proportional hazards regression analysis. Results were adjusted for age.

We identified 336 women with atypical hyperplasia. With a mean follow-up of 12.2 years, 66 breast cancers occurred in these women. Atypia conveyed an increased risk of breast cancer (SIR 4.4, 95% CI 2.4-5.6). Marked elevations in risk were seen in women with three or more foci of atypia (SIR 9.3, 95% CI 5.8-14.1) and especially for three or more foci with calcifications (SIR 12.8, 95% CI 7.8-19.7). Risk was higher in women diagnosed with atypical hyperplasia before the age of 45 (SIR 7.4, 95% CI 3.6-13.7) versus atypia diagnosed at age 45-55 (SIR 5.5, 95% CI 3.6-8) or greater than 55 (SIR 3.4, 95% CI 2.3-4.9). Risk was similar for ductal and lobular types of atypia; family history did not significantly increase risk. Breast cancer risk in women with atypia remained elevated over 20 years.

G. Analyze expression data

The profiling experiments (see III.A, III.E, and IV.E) are just getting underway.

KEY RESEARCH ACCOMPLISHMENTS

- We identified the degree of risk associated with various histiologic subtypes of BBD and the extent to which family history influences the risk of breast cancer in women with proliferative or atypical lesions (see results under Task 4F).

- We identified that centrosome amplification is seen more frequently in benign lesions having the highest relative risk of developing breast cancer and is infrequently seen in non-proliferative lesions and in proliferative lesions without atypia (see results under Task 2F).

- We identified that a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other non-atypical forms of proliferative breast disease. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer rsik similar to or greater than convential atypical ductal/lobular hyperplasias (see results under Task 4F).

- We found a marked increase in risk of breast cancer in women with three or more foci of atypia and especially for three or more foci with calcifications. Risk was higher in women diagnosed with atypical hyperplasia before age 45 (see results under Task 4F).

- We identified that mean age at BBD increased from 47.5 to 54.1 years of age over the 25 years of the study and that benign breast disease samples from the latter years of the study were more likely to show proliferative change with or without atypia, likely due to increased use of screening mammography and detection of abnormal calcifications (see results under Task 4F).

- UCSF has been successful in culturing fresh benign breast disease tissue shipped cross-country. They have reverse transcribed first and second strand cDNA using Ambion Message Amp kit. They are currently amplifying, biotinylating and in vitro transcribing the cDNA back into RNA for Affymetrix microarray analysis.

REPORTABLE OUTCOMES

1. Manuscript In Press:

   Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. Benign breast disease and breast cancer risk. *New England Journal of Medicine*; 353 (slated for 7/21/05 issue).

2. Manuscript Under Review

   Lewis J, Hartmann L, Vierkant R, Maloney S, Frost M, Allers T, Visscher D. Analysis of cancer risk among patients with papillary lesions of the breast.

3. Symposium Presentation at Department of Defense Era of Hope June 9, 2005 in Philadelphia, Pennsylvania

   Benign Breast Disease and Breast Cancer Risk: Center of Excellence Discussion

   - Benign Breast Disease: Evidence for Precursor Lesions - Lynn C. Hartmann

- Statistical Methods to Assess the Timing and Side of Breast Cancer Relative to Benign Breast Biopsies: Implications for Potential Precursor Lesions - V. Shane Pankratz
- Multifocal Atypia Confers Increased Risk of Breast Cancer - Amy C. Degnim
- Temporal Changes in Benign Breast Disease 1967 to 1991 - Karthik Ghosh

4. Poster Presentations at the Department of Defense Era of Hope June 10, 2005 in Philadelphia, Pennsylvania:
   - Degnim AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SC, Pankratz VS, Slleres TA, Lingle WL, Tlsty T, Berman H, Hartmann LC. Multifocal Atypia Confers Increased Risk of Breast Cancer
   - Ghosh K, Hartmann LC, Sellers TA, Degnim AC, Pankratz VS, Blake C, Tlsty T, Melton LJ, Visscher DW. Temporal Changes in Benign Breast Disease 1967 to 1991
   - Hartmann LC, Frost MH, Ghosh K, Degnim A, Vierkant RA, Maloney SD, Pankratz VS, Tlsty T, Blake C, Sellers TA, Lingle WL, Melton J, Visscher D Benign Breast Disease and Breast Cancer Risk
   - Hartmann LC, Degnim A, Frost MH, Vierkant RA, Maloney SD, Sellers, TA, Pankratz VS, Tlsty T, Blake C. Lingle WL, Visscher DW. Benign Breast Disease: Evidence for Precursor Lesions
   - Pankratz VS, Vierkant RA, Maloney SD, Degnim AC, Hartmann LC. Statistical Methods to Assess the Timing and Side of Breast Cancer Relative to Benign Breast Biopsies: Implications for Potential Precursor Lesions
5. Podium Presentation at annual meeting of the United States and Canadian Academy of Pathology. February 29, 2005 in San Antonio, Texas:
   Lewis, JT, Vierkant RA, Maloney SD, Hartmann LC, Visscher DW. Analysis of Cancer Risk among Patients with Papillary Lesions of the Breast
6. Podium Presentation at Society of Surgical Oncology Annual Cancer Symposium, March 3-6, 2005 in Atlanta, Georgia:
   Degnim, AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SD, Pankratz VS, Sllers TA, Lingle WL, Hartmann LC. Multifocal Atypia Confers Increased Risk of Breast Cancer
7. Poster Presentation at annual meeting of American Association for Cancer Research, April 16-20 in Anaheim, California:
   Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. Benign Breast Disease and Breast Cancer Risk in the Mayo Cohort Study

CONCLUSIONS

We have made significant progress on all three aims. Specifically we have completed the cohort follow-up by questionnaire. We have obtained and catalogued the majority of the benign and cancer tissue. Our pathologist has completed readings on the benign tissue for the entire cohort and is beginning to read the cancer tissue. We have prepared tissue slides for our test set and two other subgroups, those women for whom breast cancer occurred within 5 years of their BBD and women with atypia. In our BBD samples, we are currently examining the significance of cox-2 staining and centrosome status, and will be focusing on the activation of DNA damage pathways. UCSF has successfully cultured fresh benign tissue and has provided a composite growth curve for the initial 14 samples. We have calculated the risk of a subsequent breast cancer by age at BBD, family history and histology. We will be focusing this upcoming year on molecular markers and genetic profiling.

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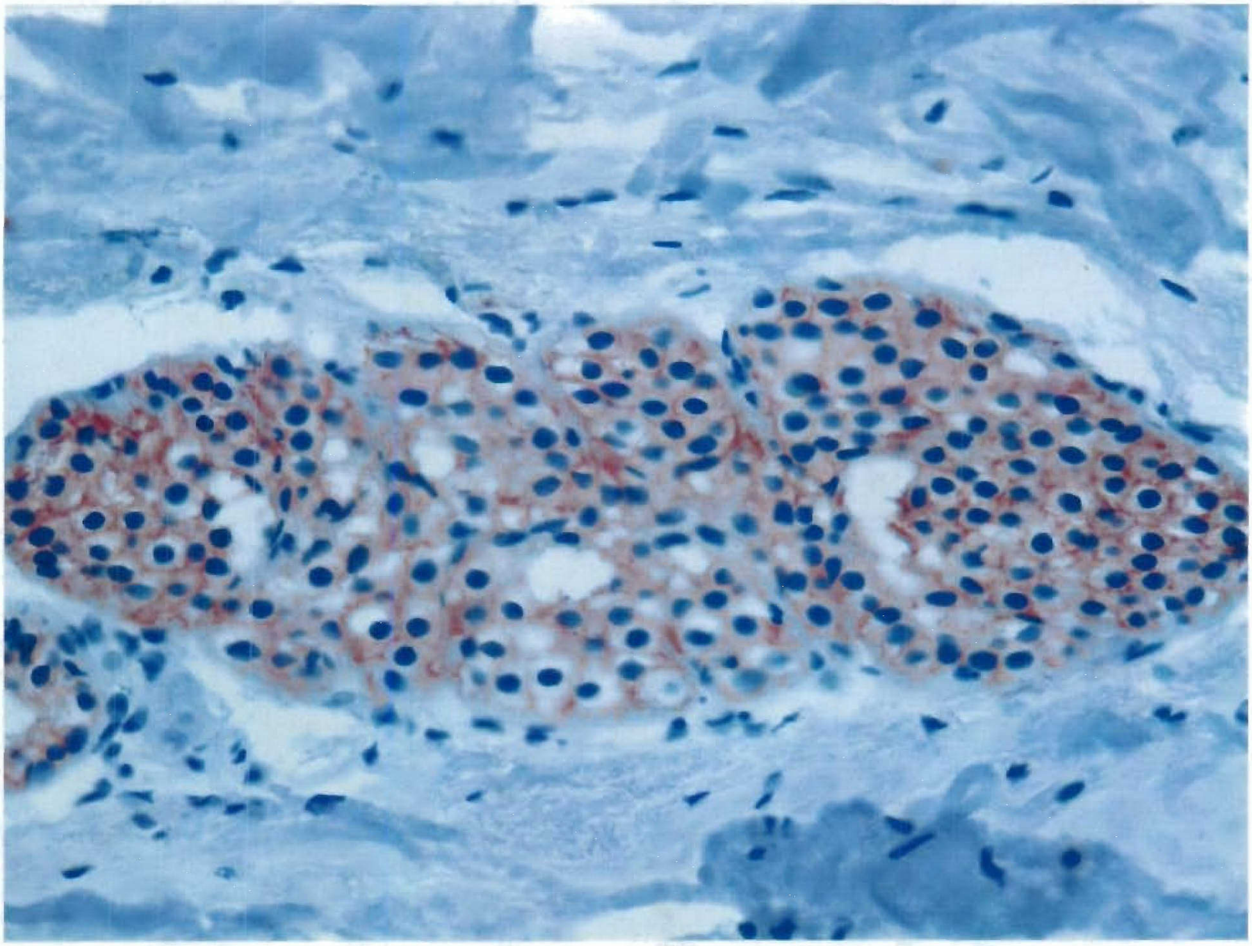
3. Shim V et al. Cyclooxygenase-2 expression is related to nuclear grade in ductal carcinoma in situ and is increased in its normal adjacent epithelium. Cancer Research 2003;63:2347-50.
4. Bartkova J et al, DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis; Nature 2005; 434:864-870.
5. Gorgoulis VG et al, Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions; Nature 2005; 434:907-912.

Appendix A. Examples of Cox-2 staining in atypica samples.



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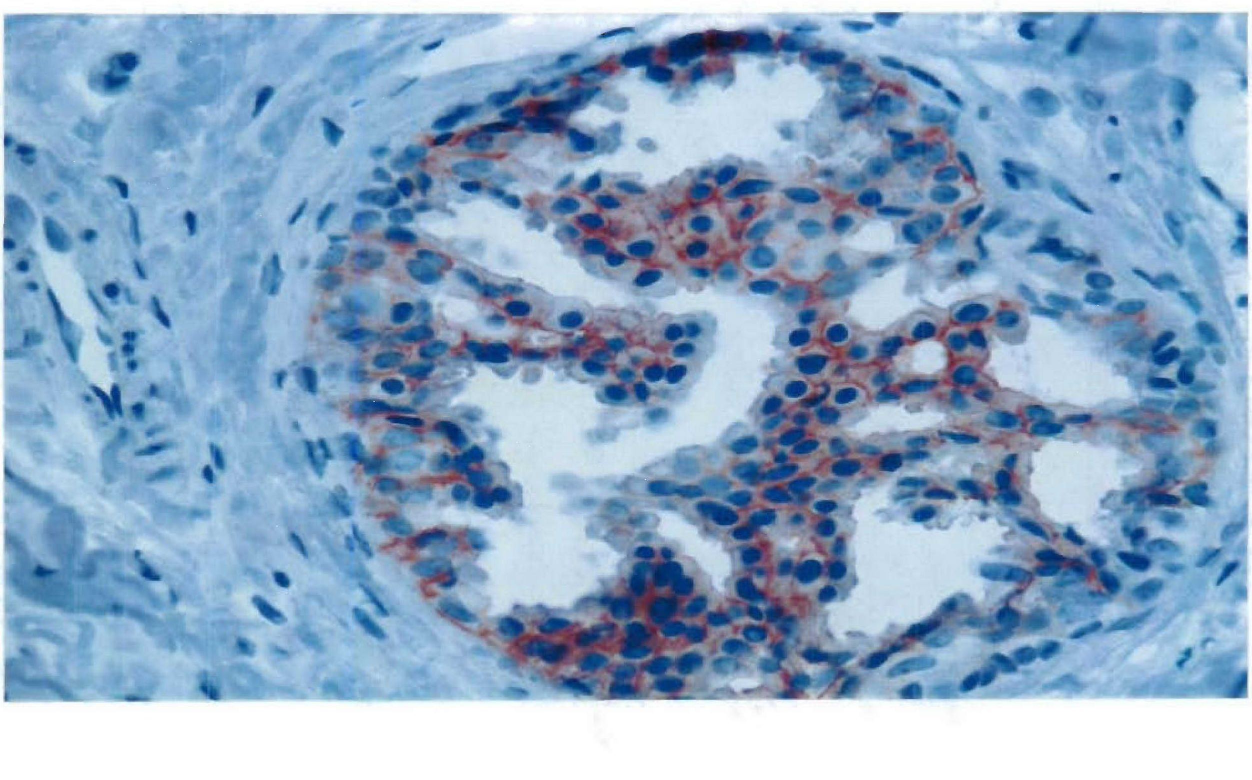

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Appendix B: *New England Journal of Medicine* galleys (confidential)

The NEW ENGLAND JOURNAL of MEDICINE

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Benign Breast Disease and the Risk of Breast Cancer

Lynn C. Hartmann, M.D., Thomas A. Sellers, Ph.D., Marlene H. Frost, Ph.D., Wilma L. Lingle, Ph.D., Amy C. Degnim, M.D., Karthik Ghosh, M.D., Robert A. Vierkant, M.A.S., Shaun D. Maloney, B.A., V. Shane Pankratz, Ph.D., David W. Hillman, M.S., Vera J. Suman, Ph.D., Jo Johnson, R.N., Cassann Blake, M.D., Thea Tlsty, Ph.D., Celine M. Vachon, Ph.D., L. Joseph Melton III, M.D., and Daniel W. Visscher, M.D.

ABSTRACT

BACKGROUND

Benign breast disease is an important risk factor for breast cancer. We studied a large group of women with benign breast disease to obtain reliable estimates of this risk.

METHODS

We identified all women who received a diagnosis of benign breast disease at the Mayo Clinic between 1967 and 1991. Breast-cancer events were obtained from medical records and questionnaires. To estimate relative risks, we compared the number of observed breast cancers with the number expected on the basis of the rates of breast cancer in the Iowa Surveillance, Epidemiology, and End Results registry.

RESULTS

We followed 9087 women for a median of 15 years. The histologic findings were nonproliferative lesions in 67 percent of women, proliferative lesions without atypia in 30 percent, and atypical hyperplasia in 4 percent. To date, 707 breast cancers have developed. The relative risk of breast cancer for the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95 percent confidence interval, 3.26 to 5.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for proliferative changes without atypia and of 1.27 (95 percent confidence interval, 1.15 to 1.41) for nonproliferative lesions. The strength of the family history of breast cancer, available for 4808 women, was a risk factor that was independent of histologic findings. No increased risk was found among women with no family history and nonproliferative findings. In the first 10 years after the initial biopsy, an excess of cancers occurred in the same breast, especially in women with atypia.

CONCLUSIONS

Risk factors for breast cancer after the diagnosis of benign breast disease include the histologic classification of a benign breast lesion and a family history of breast cancer.

From the Divisions of Medical Oncology (L.C.H., M.H.F., J.J.), Experimental Pathology (W.L.L.), General Surgery (A.C.D.), General Internal Medicine (K.G.), Biostatistics (R.A.V., S.D.M., V.S.P., D.W.H., V.J.S.), Epidemiology (C.M.V., L.J.M.), and Anatomic Pathology (D.W.V.), Mayo Clinic College of Medicine, Rochester, Minn.; H. Lee Moffitt Cancer Center and Research Institute, Tampa, Fla. (T.A.S.); Wayne State University, Detroit (C.B.); and the University of California, San Francisco, San Francisco (T.T.). Address reprint requests to Dr. Hartmann at Mayo Clinic College of Medicine, Rochester, MN 55905.

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Benign breast disease is an important risk factor for a later breast cancer, which can develop in either breast.[1] It encompasses a spectrum of histologic entities, usually subdivided into nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasias, with an increased risk of breast cancer associated with proliferative or atypical lesions.[2-4] The identification of benign breast disease has become more common as the use of mammography has increased, and thus, having accurate risk estimates for women who receive this diagnosis is imperative.

Important questions remain, however, about the degree of risk associated with the common nonproliferative benign entities and the extent to which family history influences the risk of breast cancer in women with proliferative or atypical lesions. Dupont and Page found that women with nonproliferative disease did not have an increased risk of a later breast cancer.[2] By contrast, a companion study to the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1) found a relative risk of 1.6 for women who received a diagnosis of a "lower category" of benign breast disease.[5] A limitation of the NSABP study, however, was the lack of central pathological review.

Another major question concerns the possible interplay between atypia and a family history of breast cancer. The Dupont and Page study found that women with atypia and a family history had 11 times the risk of those with nonproliferative lesions and no family history.[2] However, two other major studies of benign breast disease[6,7] did not find a significant interaction between atypia and family history. The duration of increased risk after a finding of benign disease on biopsy is also uncertain.[2,4,8]

Studies of benign breast disease can also clarify whether there is a continuum of breast alterations that culminates in breast cancer. However, it remains unclear which of the benign entities are actual precursors and which reflect a background of increased risk involving all breast tissue in a woman. Determining the extent of agreement between the side (right or left) of the benign lesion and the subsequent breast cancer is one means of assessing these issues.

To investigate these questions, we studied 9087 women with benign breast disease for whom we had follow-up data on breast-cancer events. This cohort has been followed for a median of 15 years, and 707 breast cancers have developed, making this, to our knowledge, one of the largest such studies of its kind. We report on the risk of breast cancer according to histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history. We also recorded the side of the cancer (ipsilateral or contralateral) and the time to the diagnosis of cancer.

METHODS

STUDY POPULATION

We accessed data from the Mayo Clinic Surgical Index and Pathology Index to identify all women 18 to 85 years of age who had undergone surgical excision of a benign breast lesion during the 25-year period from January 1, 1967, through December 31, 1991. For women who had more than one biopsy during this period, we used the first sample. The original list contained 12,132 women, but we excluded 1,047 women for any of the following: a diagnosis of breast cancer or lobular carcinoma in situ at, before, or within six months after the biopsy of the benign lesion; mastectomy (unilateral or bilateral) or breast reduction at or before biopsy; or refusal to allow use of their medical records for research.[9] This left 11,085 women. Of these, 1053 (9.5 percent) had no follow-up information after the biopsy. Thus, a total of 10,032 women met our criteria for study entry and had follow-up information. Of these, 945 women had unusable or unavailable biopsy specimens of the benign lesion. The remaining group of 9087 women constitutes our study cohort. The relative risks of breast cancer (described below) did not differ significantly between the 10,032 women who met our criteria and the 9087 women who made up the study cohort (1.59 and 1.56, respectively).

FAMILY HISTORY AND FOLLOW-UP

A questionnaire designed for this study was used to obtain information about family history and other possible risk factors for breast cancer. Thus, our family-history data were obtained at the time of follow-up contact. We categorized family history as none, weak, or strong. The criteria for a strong family history were as follows: at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history of breast cancer was categorized as weak. The questionnaire also asked about breast-cancer occurrences. Follow-up for breast-cancer events was also obtained through the comprehensive (inpatient and outpatient) Mayo medical

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record. Questionnaire information was available for 5619 women (61.8 percent). Of the questionnaires, 604 (10.7 percent) were completed by proxy (the next of kin of a deceased patient). As of August 1, 2004, 7260 (79.9 percent) members of the cohort were still alive. All protocol procedures and patient-contact materials were reviewed and approved by the institutional review board of the Mayo Clinic; returning the contact materials was considered implied consent.

HISTOLOGY

Stored hematoxylin-and-eosin–stained sections from each participant were evaluated by a breast pathologist who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al.[2,10] into the following categories: nonproliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes with atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) (Fig. 1).[2,10] Biopsy specimens were designated as having proliferative fibrocystic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or sclerosing adenosis. Cysts, fibroadenoma, or columnar changes were considered nonproliferative unless they also contained one of the lesions denoted above.

STATISTICAL ANALYSIS

The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or last contact. We estimated relative risks on the basis of standardized incidence ratios (SIRs), dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman's follow-up into five-year age and calendar-peri-

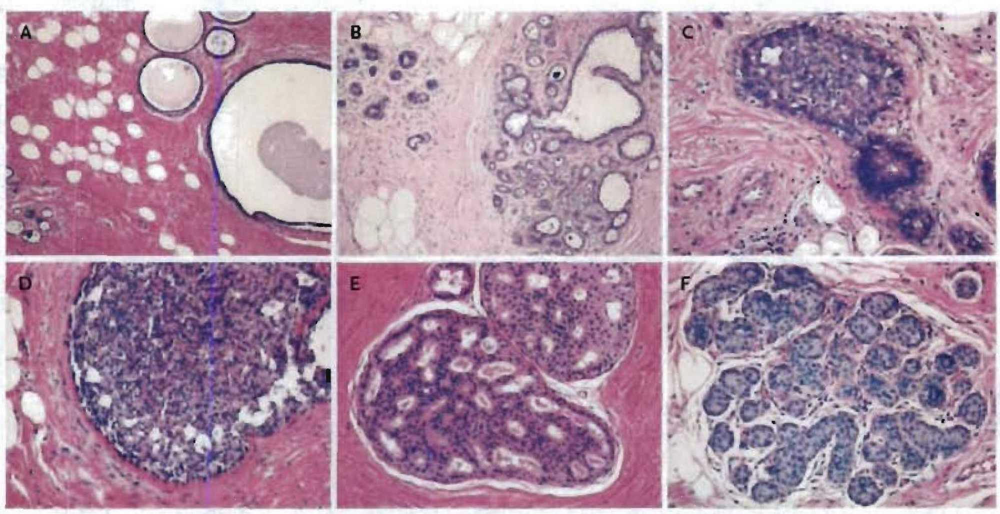

Figure 1. Histopathological Appearance of Benign Breast Disease (Hematoxylin and Eosin).

Panel A shows nonproliferative fibrocystic changes: the architecture of the terminal-duct lobular unit is distorted by the formation of microcysts, associated with interlobular fibrosis. Panel B shows proliferative hyperplasia without atypia. This is adenoma, a distinctive form of hyperplasia characterized by the proliferation of lobular acini, forming crowded gland-like structures. For comparison, a normal lobule is on the left side. Panel C also shows proliferative hyperplasia without atypia. This is moderate ductal hyperplasia, which is characterized by a duct that is partially distended by hyperplastic epithelium within the lumen. Panel D again shows proliferative hyperplasia without atypia, but this is florid ductal hyperplasia: the involved duct is greatly expanded by a crowded, jumbled-appearing, epithelial proliferation. Panel E shows atypical ductal hyperplasia: these proliferations are characterized by a combination of architectural complexity with partially formed secondary lumens and mild nuclear hyperchromasia in the component-cell population. Panel F shows atypical lobular hyperplasia: monotonous cells fill the lumens of partially distended acini in this terminal-duct lobular unit.

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od categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80 percent of cohort members reside in the upper Midwest). Over 95 percent of our cohort was white, equivalent to that reported in Iowa census data during the study period.[11] In the SIR analyses, we considered the time since the original biopsy as a time-dependent variable and all other factors as fixed.

Associations between the risk of breast cancer and histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history of cancer, as well as pairwise combinations of these variables, were examined with the use of Cox proportional-hazards regression analysis. The main effects for each categorized variable and the corresponding interaction terms were included in each model, and the statistical significance of each interaction was evaluated with the use of a multiple degree-of-freedom likelihood-ratio test.

We studied ipsilateral and contralateral breast cancer as a function of the time since biopsy by estimating the relative risk of cancer in the same as compared with the opposite breast for five-year intervals. When calculating the incidence of ipsilat-

Table 1. Characteristics of the Women According to the Histologic Category of Benign Breast Disease.*

Characteristic	All Women (N=9087)	Nonproliferative Disease (N=6061)	Proliferative Disease without Atypia (N=2690)	Atypical Hyperplasia (N=336)
Percentage of total	100.0	66.7	29.6	3.7
Age at biopsy — no. of women (%)				
<40 yr	1841 (20.3)	1500 (24.7)	323 (12.0)	18 (5.4)
40–49 yr	2474 (27.2)	1621 (26.7)	770 (28.6)	83 (24.7)
50–59 yr	2145 (23.6)	1297 (21.4)	759 (28.2)	89 (26.5)
60–69 yr	1639 (18.0)	1034 (17.1)	522 (19.4)	83 (24.7)
≥70 yr	988 (10.9)	609 (10.0)	316 (11.7)	63 (18.8)
Mean age at biopsy — yr	51.4±14.3	49.9±14.8	53.9±12.6	57.8±12.3
Menopausal status at biopsy — no. of women (%)†				
Premenopausal (<45 yr)	2948 (32.4)	2246 (37.1)	652 (24.2)	50 (14.9)
Perimenopausal (45–55 yr)	2583 (28.4)	1610 (26.6)	871 (32.4)	102 (30.4)
Postmenopausal (>55 yr)	3556 (39.1)	2205 (36.4)	1167 (43.4)	184 (54.8)
Family history of breast cancer — no. of women (%)				
Unknown	4279 (47.1)	2970 (49.0)	1170 (43.5)	139 (41.4)
Known	4808 (52.9)	3091 (51.0)	1520 (56.5)	197 (58.6)
None	2668 (55.5)	1735 (56.1)	831 (54.7)	102 (51.8)
Weak	1174 (24.4)	756 (24.5)	378 (24.9)	40 (20.3)
Strong	966 (20.1)	600 (19.4)	311 (20.5)	55 (27.9)
Breast-cancer status — no. of women (%)				
Negative	8380 (92.2)	5682 (93.7)	2426 (90.2)	272 (81.0)
Positive	707 (7.8)	379 (6.3)	264 (9.8)	64 (19.0)
Vital status — no. of women (%)				
Deceased	1827 (20.1)	1172 (19.3)	566 (21.0)	89 (26.5)
Alive	7260 (79.9)	4889 (80.7)	2124 (79.0)	247 (73.5)

* Plus–minus values are means ±SD.

† Menopausal status was categorized according to the age at breast biopsy.

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eral cancer, we censored follow-up on women with contralateral cancer after the date of diagnosis. Similarly, when calculating the incidence of contralateral cancer, we censored follow-up on women with ipsilateral cancer after the date of diagnosis. Data on women missing information on the side of the cancer or women who had bilateral biopsies or cancer were not included in these analyses. This approach yields identical numbers of person-years for each type of event. As a result, the length of follow-up is no longer a factor in the analysis and the relative risks are equivalent to simple ratios of event counts. We therefore used properties of the binomial distribution to obtain exact P values and 95 percent confidence intervals for these relative risks.[12] Statistical tests were two-sided, and analyses were conducted with the use of SAS (SAS) and Splus (Insightful) software.

RESULTS

CHARACTERISTICS OF PATIENTS AND PATHOLOGICAL SPECIMENS

The final cohort consisted of 9087 women with benign breast disease as determined by open surgical biopsy. Table 1 shows the age at the time of the biopsy, likely menopausal status on the basis of age, and the strength of the family history of breast cancer according to the histologic findings for the benign lesion. The broad histologic classifications included nonproliferative disease in 6061 (66.7 percent), proliferative disease without atypia in 2690 (29.6 percent), and atypical hyperplasia in 336 (3.7 percent). Figure 1 shows examples of these lesions. The mean age was 51.4 years, but women with nonproliferative findings were slightly younger, whereas those with atypia tended to be older (mean age,

Table 2. Risk Factors for Breast Cancer after the Diagnosis of Benign Breast Disease.*

Characteristic	No. of Women	Person-Years	No. of Observed Events	No. of Expected Events	Relative Risk (95% CI)†
Overall	9087	144,881	707	453.0	1.56 (1.45–1.68)
Age at diagnosis of benign breast disease					
<30 yr	726	13,593	21	11.5	1.83 (1.13–2.80)
30–39 yr	1115	20,169	71	38.3	1.85 (1.45–2.34)
40–49 yr	2474	45,780	212	136.3	1.56 (1.35–1.78)
50–59 yr	2145	34,100	196	125.9	1.56 (1.35–1.79)
60–69 yr	1639	21,364	142	94.5	1.50 (1.27–1.77)
≥70 yr	988	9,874	65	46.6	1.40 (1.08–1.78)
Menopausal status‡					
Premenopausal (age <45 yr)	2948	54,419	169	106.1	1.59 (1.36–1.85)
Perimenopausal (age 45–55 yr)	2583	45,872	245	153.4	1.60 (1.40–1.81)
Postmenopausal (age >55 yr)	3556	44,590	293	193.6	1.51 (1.35–1.70)
Histologic findings					
Nonproliferative disease	6061	99,109	379	297.7	1.27 (1.15–1.41)
Proliferative disease without atypia	2690	41,610	264	140.2	1.88 (1.66–2.12)
Atypical hyperplasia	336	4,161	64	15.1	4.24 (3.26–5.41)
Family history of breast cancer§					
None	2668	44,974	171	145.4	1.18 (1.01–1.37)
Weak	1174	21,472	94	65.9	1.43 (1.15–1.75)
Strong	966	18,087	110	57.0	1.93 (1.58–2.32)

* Numbers of women, person-years, and events may not sum to overall totals because of rounding.
† The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval.
‡ Menopausal status was categorized according to the age at breast biopsy.
§ Information on family history was available for 4808 of the 9087 women.

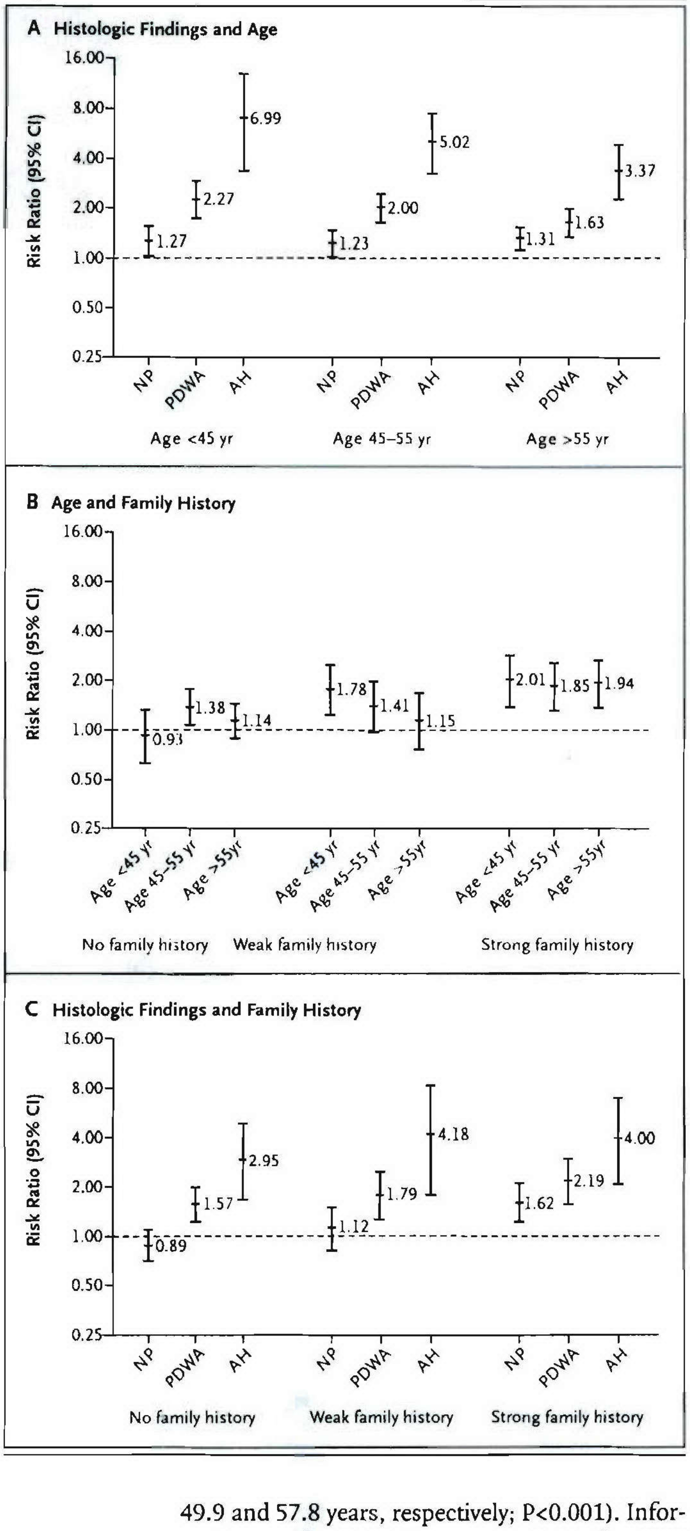

Figure 2. Risk-Factor Interaction Profiles for Benign Breast Disease, Comparing the Number of Events Observed with the Number Expected.

Expected events account for age and calendar period and are calculated with the use of Iowa SEER rates. CI denotes confidence interval, NP nonproliferative disease, PDWA proliferative disease without atypia, and AH atypical hyperplasia.

49.9 and 57.8 years, respectively; P<0.001). Information on family history was available for 4808 women and was negative in 2668 (55.5 percent), weakly positive in 1174 (24.4 percent), and strongly positive in 966 (20.1 percent). More women with atypia than without atypia had a strong family history of breast cancer (27.9 percent vs. 19.8 percent, P=0.06). The risk of cancer was highest in the group with atypia: breast cancer developed in 64 of the 336 women (19.0 percent).

FEATURES OF BENIGN BREAST DISEASE AND SUBSEQUENT RISK OF BREAST CANCER

Patients in the cohort were followed for a median of 15 years. A total of 1827 women (20.1 percent) had died and 7260 (79.9 percent) were alive as of August 2004. We have documented 707 breast cancers to date. The median time from the original biopsy to the diagnosis of breast cancer was 10.7 years. Table 2 shows the estimated relative risks of breast cancer associated with the age at the initial biopsy, the strength of the family history, menopausal status, and histologic findings of the biopsy, as compared with expected population-based incidence. The estimated relative risk of breast cancer in the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68). The risk was inversely associated with the age at biopsy, with younger women having a greater risk than older women. The type of benign breast disease identified at biopsy was a major predictor of risk. Atypical hyperplasia had a relative risk of 4.24 (95 percent confidence interval, 3.26 to 5.41), proliferative disease without atypia had a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12), and nonproliferative lesions had a relative risk of 1.27 (95 percent confidence interval, 1.15 to 1.41). Family history was an independent risk factor. For women with no known family history of breast cancer, the relative risk was only 1.18 (95 percent confidence interval, 1.01 to 1.37), as compared with 1.43 (95 percent confidence interval, 1.15 to 1.75) for women with a weak family history and 1.93 (95 percent confidence interval, 1.58 to 2.32) for those with a strong family history.

Figure 2 shows possible interactions between pairs of the major risk factors of age, histologic findings, and family history. No significant interactions were observed between age and family history or between histologic findings and family history, including atypia and family history. However, there was a significant interaction between age and histologic findings (P=0.05): the risk of breast cancer was 6.99 times the expected risk among women who received a diagnosis of atypia before the age of 45 years; the risk was 5.02 times the expected risk when the atypia was diagnosed between the ages of 45 and 55 years and 3.37 times the expected risk when it was diagnosed after the age of 55 years. An important finding was that for women with nonproliferative disease and no family history or a weak family history, there was no increase in the risk of breast cancer.

TIME COURSE AND SIDE OF BREAST CANCER AFTER BENIGN BREAST DISEASE

Figure 3 shows the observed and expected numbers of cancers at five-year intervals. The excess risk persisted for at least 25 years after the initial biopsy and perhaps for 30 years or more, but accuracy was low after 25 years. Figure 4 shows a further breakdown of breast cancers into ipsilateral or contralateral according to the histologic findings in the benign lesion. Of the 616 unilateral cancers, 342 (55.5 percent) developed in the same breast as the initial biopsy and 274 (44.5 percent) developed in the contralateral breast. In the remaining 91 cases, there were bilateral events, either benign or malignant, or information on the side of the cancer was missing. During the first 10 years, there was an excess of ipsilateral cancers, with relative risks of ipsilateral as compared with contralateral cancer of 1.88 (95 percent confidence interval, 1.33 to 2.64) for years 0 through 5 and 1.34 (95 percent confidence interval, 0.96 to 1.85) for years 6 through 10. The 35 women with atypia in whom breast cancer developed within 10 years after the initial biopsy were 2.5 times as likely (P=0.02) to have the cancer in the same breast as in the opposite breast.

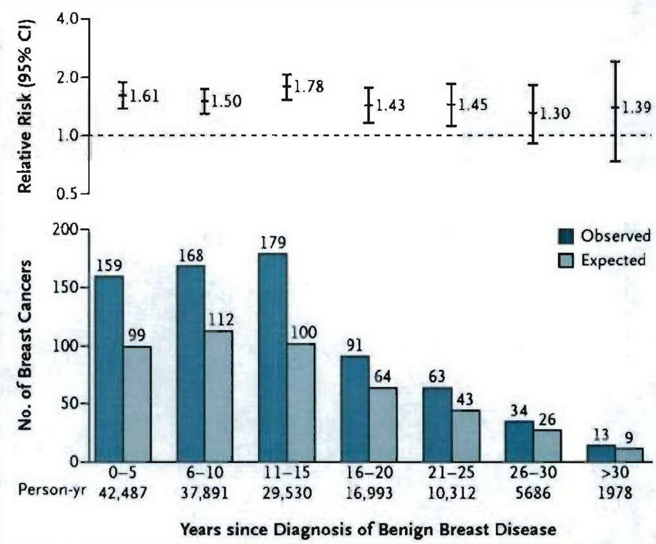

Figure 3. The Number of Breast Cancers Observed as Compared with the Number Expected over Time.

Expected events account for age and calendar period and are calculated with the use of Iowa SEER rates. CI denotes confidence interval.

DISCUSSION

Retrospective and prospective studies have shown a relative risk of breast cancer of 1.5 to 1.6 for women with benign breast disease as compared with women in the general population.[2,5-7,13-21] The histologic appearance of the benign lesion is a major determinant of risk, yet not all large studies have had access to tissue for re-review. Our investigation was based on a single-institution resource with long-term and complete follow-up for cancer events. All samples containing the benign lesion were read by a breast pathologist who applied current histologic classifications. More than 700 breast cancers developed in this cohort, giving our study good statistical power. The relative risk of breast cancer for our cohort overall was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after the initial biopsy.

The histologic appearance of the benign lesion is strongly associated with the risk of breast cancer. For biopsies with nonproliferative findings, the relative risk was 1.27 (95 percent confidence interval, 1.15 to 1.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for findings of proliferative changes but no atypia and of 4.24 (95 percent confidence interval, 3.26 to 5.41) for a finding of atypical hyperplasia. When the family history is known, risk profiles can be refined. For women with nonproliferative findings and no family history or a weak family history of breast cancer, we observed no increased risk. This finding is important, because a sizable proportion

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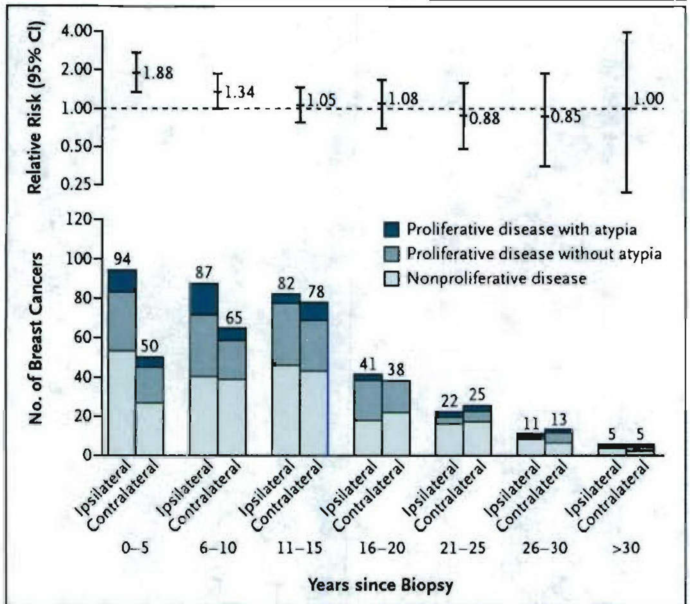

Figure 4. Comparison of the Number of Ipsilateral Breast Cancers with the Number of Contralateral Breast Cancers over Time, According to the Histologic Appearance of Benign Breast Disease.

Results are shown for 616 cancers (342 ipsilateral and 274 contralateral cancers). The remaining 91 cases include women with bilateral benign or malignant lesions or for whom the side of the benign or malignant lesion was unknown. CI denotes confidence interval.

of women with benign breast disease are in this group (52 percent of our cohort with a known family-history status). Dupont and Page made a similar observation in their 1985 report.[2] However, a recent NSABP study found a significantly increased risk of breast cancer among women with lower-category benign breast disease, including nonproliferative disease.[5] In the NSABP P1 trial, which included more than 13,000 women, 1376 had a breast biopsy with benign findings over a mean follow-up period of 79 months. Breast cancer developed in 47 of these women. On the basis of pathology reports from contributing centers, the investigators reported a relative risk of 1.6 among women with lower category findings on breast biopsy as compared with P1 participants who did not undergo a breast biopsy.[5]

In our study, the degree of family history was an independent risk factor. In women with a strong family history of breast cancer, even nonproliferative findings were associated with a risk ratio of 1.62. This subgroup may parallel the high-risk NSABP cohort.[5] Women with atypia are at significantly increased risk, but a family history did not significantly modify the atypia-associated risk (Fig. 2). The risk was four times the expected risk among women with atypia and a family history of breast cancer, regardless of the degree of their family history; among women with atypia without a family history of breast cancer, the risk ratio was 2.95 (95 percent confidence interval, 1.65 to 4.87).

The age at the diagnosis of benign breast disease appears to modify the risks related to the histologic appearance of benign breast disease. The presence of atypia in women under 45 years of age conveyed twice the risk observed among women over 55 years of age (6.99 and 3.37, respectively), which might relate, in part, to menopausal status. The Breast Cancer Detection and Demonstration Project showed that the risk of breast cancer among premenopausal women with atypia was elevated by a factor of 12.0 (95 percent confidence interval, 2.0 to 68.0), as compared with 3.3 among postmenopausal women with atypia (95 percent confidence interval, 1.1 to 10.0), but the numbers of patients in the study were small.[22] The Nurses Health Study also showed an increased risk of breast cancer among premenopausal women with atypia.[7] However, in the NSABP study of women with lower categories of benign breast disease, the risk of breast cancer was greatest among postmenopausal women.[5]

Understanding the risk associated with benign breast disease is important because the increasing use of mammography has increased the frequency of breast biopsies, most of which yield benign findings. In a retrospective study of women undergoing annual mammographic screening, Elmore et al. found that 18.6 percent of women underwent a biopsy after 10 screening mammograms.[23] The use of hormone therapy may also affect the frequency of breast biopsies. Chlebowski et al., reporting for the Women's Health Initiative investigators, found that relatively short-term therapy with estrogen plus progestin increased the percentage of women with abnormal mammograms, a major indicator for breast biopsy.[24]

Regarding the possibility of malignant precursors within benign breast disease, we have information on the side and the time to breast cancer for 616 unilateral events. An excess of breast cancers occurred in the same breast during the first years of follow-up, especially in women with atypia (Fig. 4). This finding suggests that precursors to breast cancer exist in benign breast disease. Work in model systems of early steps in mammary carcinogenesis

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has identified alterations in key regulatory indicators that can be studied in selected benign breast lesions.[25,26]

In summary, our study shows that histologic features, the age at biopsy, and the degree of family history are major determinants of the risk of breast cancer after the diagnosis of benign breast disease. We found no increased risk among women with nonproliferative lesions, unless a strong family history was present. No significant interaction between atypia and family history was apparent. The excess risk of cancer in the ipsilateral breast in the first 10 years after the diagnosis of benign breast disease, especially in women with atypia, points to the presence of precursors in some women.

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Appendix C: Presentation Abstracts

BENIGN BREAST DISEASE AND BREAST CANCER RISK

LC Hartmann[1], MH Frost[1], K Ghosh[1], A Degnim[1], RA Vierkant[1], SD Maloney[1], VS Pankratz[1], T Tlsty[2], C Blake[3], TA Sellers[4], WL Lingle[1], LJ Melton[1], D Visscher[1]
Mayo Clinic Cancer Center[1], Rochester, MN; University of California[2], San Francisco, CA; Wayne State University[3], Detroit, MI; Moffitt Cancer Center[4], Tampa, FL. E-mail: hartmann.lynn@mayo.edu

Background: Benign breast disease (BBD) represents a significant risk factor for a later breast cancer that can develop in either breast. Questions remain about the degree of risk associated with non-proliferative findings and the degree of interaction between atypia and family history. Having accurate risk estimates is essential to counsel women properly regarding surveillance and risk reduction strategies.

Methods: The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. Our study pathologist (DV) reviewed and classified all benign lesions. Medical records and a study-specific questionnaire were used to collect risk factor data and to identify subsequent breast cancers (BC). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

Results: This 25-year cohort includes 9,087 women with 144,881 person years of follow-up (median 15 yrs). The mean age at BBD was 51.4 years. Non-proliferative disease was found in 66%, proliferative disease without atypia in 30% and atypia (atypical ductal hyperplasia or atypical lobular hyperplasia) in 4%. Thus far, 707 women are known to have developed BC, at a median of 10.7 years after their BBD.

The overall relative risk for breast cancer in our cohort is 1.56 (95% CI 1.45 -1.68). Benign histology was a major predictor of risk. Atypical hyperplasia conveyed a relative risk of 4.24 (3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with proliferative disease without atypia and 1.27 (1.15 – 1.41) for non-proliferative lesions. Knowledge of family history allowed further refinement of risk estimates. For women with no family history, the relative risk was 1.18 (1.01 – 1.37) compared to 1.43 (1.15 – 1.75) for women with a weak family history, and 1.93 (1.58 – 2.32) for those with a strong family history. For women with non-proliferative findings and no or weak family history, there was no increased risk. We did not see an interaction between atypia and family history. Women with atypia and no family history had a RR of 2.95 (1.65 – 4.87) vs 4.18 (1.80 – 8.23) for those with a weak family history and 4.0 (2.07 – 6.99) for those with a strong family history. Risk of BC was inversely associated with age at benign biopsy, with younger women demonstrating greater risk than older women (RR for age < 30 = 1.83 vs RR 1.40 for age ≥ 70).

Conclusions: Benign breast disease is a major risk factor for a later breast cancer. Within BBD, age at BBD, family history and histology are major predictors of subsequent risk.

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BENIGN BREAST DISEASE: EVIDENCE FOR PRECURSOR LESIONS

LC Hartmann, A Degnim, MH Frost, RA Vierkant, SD Maloney, TA Sellers, VS Pankratz, T Tlsty, C Blake, WL Lingle, DW Visscher
Mayo Clinic and Mayo Foundation, Rochester, MN; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; University of California, San Francisco, CA; Wayne State University, Detroit, MI. E-mail: hartmann.lynn@mayo.edu

Background: Benign breast disease (BBD) represents a significant risk factor for a later breast cancer (BC) that can occur in either breast. Besides aiding in risk prediction, BBD provides a possible window into a continuum of alterations culminating in BC. Information about time to and side of BC after BBD has not been available for most prior studies of BBD. Such information can help distinguish possible precursor lesions from markers of increased risk.

Methods: We used the Mayo Clinic Surgical Index to identify women ages 18-85 who had BBD between 1-1-67 and 12-31-91. The benign H&E-stained sections were evaluated by our study pathologist (DV). Biopsies were classified into: 1) non-proliferative changes, 2) proliferative changes without atypia (PDWA), and 3) atypical hyperplasia (AH). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

Results: This cohort consists of 9087 women who have been followed for a median of 15 years (person years 144, 881). The benign histologies include: non-proliferative [n=6061 (66%)], PDWA [n=2690 (30%)] and AH [n=336 (4%)]. 707 breast cancers have occurred to date. The overall relative risk for breast cancer for the entire cohort is 1.56 (95% CI 1.45 – 1.68). Benign histology was a major predictor of risk. AH conveyed a relative risk of 4.24 (95% CI 3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with PDWA and 1.27 (1.15 – 1.41) for non-proliferative lesions. The table shows median years to BC and side of BC by histologic category for those women who developed BC. There is a greater tendency for BC to develop sooner (p=0.03) and in the ipsilateral breast in women whose BBD contained increasing degrees of proliferation and atypia—consistent with the presence of precursors in these higher risk entities.

Conclusion: Information about side of BC and time to BC in studies of BBD can help to identify probable precursor lesions. Studies based in these lesions can guide our understanding of molecular risk and molecular carcinogenesis.

Sidedness and Timing of Breast Cancers after BBD

Benign Histology	# of BCs*	Median Yrs to BC (1st -3rd quartile)	Side of BC* Same (n,%)	Opposite (n,%)
Non-proliferative	379	10.7 (5.4-16.4)	185 (54)	156 (46)
PDWA	264	11.0 (5.8-16.0)	123 (56)	96 (44)
AH	64	9.3 (5.7-14.5)	34 (61)	22 (39)

* cancers where both BBD and BC were unilateral events and side for both was known

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Temporal Changes in Benign Breast Disease 1967-1991

Ghosh K, Hartmann LC, Sellers TA, Degnim AC, Pankratz VS, Blake C, Tlsty T, Melton LJ, Visscher DW

Background: Women with benign breast disease (BBD) are at increased risk of breast cancer (BC). The classic study of BBD by Dupont and Page enrolled women with biopsies in the 1950s-1960s. We sought to assess changes in the nature of BBD over time, utilizing a 25-year cohort of BBD from the late 1960s to the early 1990s.

Methods: Utilizing the Mayo Clinic Surgical and Pathology Indices, women ages 18 to 85 who had benign excisional breast biopsy between January 1, 1967 and December 31, 1991 were identified. The clinical outcome of BC was the end-point for follow-up for the 'cases' and was determined using the Mayo medical record and questionnaire information sent to study participants. Our breast pathologist (DV), blinded to both the initial diagnosis and clinical outcome, performed pathology review.

Results: The study cohort consisted of 9,087 women with benign breast disease from 1967 through 1991. The median follow-up was 15 years. 8% of the women with benign breast disease in the cohort developed subsequent breast cancer. Assessing the proportion of subjects by the year of biopsy revealed a gradual increase in the frequency of benign breast biopsies with each 5-year interval from 1967 to 1991. The mean age at biopsy increased from 47.5 in the 1960s to 54.1 in the late 1980s. The proportion of benign breast disease with atypical hyperplasia increased from 1.3% in the early part of the study to 6% in the latter part of the study. Similarly, the proportion of proliferative disease without atypia also increased from 24.1% in the early 70s to 34.8% in the late 80s. The risk of breast cancer for women diagnosed with BBD was 1.56 (95%CI: 1.45-1.68) and showed a slight decreasing trend over time. Family history was positive for breast cancer for 44.5% of the cohort with known family history and 20% had a strong family history.

Conclusions: This study provides data regarding the changing nature of BBD. The number of women in each 5-year period increased, likely due to growth of clinical practice at Mayo Clinic but may also reflect increased adoption of screening mammography. Within each time-frame, there were over 100 cases of BC, but the proportion of 'cases' to 'non-cases' decreased with decreasing 'years of risk' for women in the latter part of the study. Mean age at biopsy increased from 47.5 to 54.1, and BBD samples from the latter years of the study were more likely to show proliferative change with or without atypia, again likely due to increased use of screening mammography and detection of abnormal calcifications. The stable proportion of women with positive family history, about 20% whom had a strong family history, is consistent with general breast cancer awareness and screening practices in this population.

Statistical Methods To Assess The Timing And Side Of Breast Cancer Relative To Benign Breast Biopsies: Implications For Potential Precursor Lesions

V.S. Pankratz, R.A. Vierkant, S.D. Maloney, A.C. Degnim, L.C. Hartmann
Mayo Clinic and Mayo Foundation

<u>Introduction</u>: Benign breast disease is an important predictor of risk for breast cancer. It may also provide information about a continuum of benign breast alterations culminating in breast cancer. The agreement between side of the benign lesion and subsequent breast cancer provides one means of obtaining evidence for the presence of precursors. However, little data have been reported describing the concordance between side of the benign lesion and the cancer. Also, methods to assess the evidence of this concordance, particularly with regarding the time interval between benign lesion and breast cancer are lacking.

<u>Methods</u>: Extensive follow-up data were obtained from a consecutive series of women undergoing an open breast biopsy with benign findings from 1967 through 1991, including the timing of subsequent breast cancers and the side(s) of benign biopsy and cancer development. A variety of methods to assess concordance between benign lesions and breast cancers were explored. These ranged from the simple (e.g. chi-square tests) to the complex (e.g. survival models). Ultimately, we estimated the relative risk of cancer in the same vs. the opposite breast for five-year time intervals using a survival analysis approach by computing the relative incidence of ipsilateral and contralateral cancers. We calculated the incidence for each of these categories using two observations per person and censoring for the type of cancer that did not occur. Using this method, the relative risks are equivalent to ratios of observed events, as the approach yields identical person years for each event type. We capitalized on this and used properties of the binomial distribution to obtain exact p-values and 95% confidence intervals for these relative risks.

<u>Results</u>: The study has so far followed 9087 eligible women for 144,881 person-years (median 15 years), and 707 breast cancers have been observed to date. 91 of these cases were either missing side information, or had bilateral biopsies or cancers. Most of the unilateral events, 342 of 616 (56%), developed in the same breast as the benign biopsy. During the first ten years, there was an excess of ipsilateral cancers, with relative risks for ipsilateral vs. contralateral of 1.88 and 1.34 for years 0-5 and 6-10, respectively. Additionally, the 35 women with atypia who developed breast cancer within 10 years of their benign biopsy were 2.5 times more likely ($p=0.02$) to develop cancer in the same breast vs. the opposite breast.

<u>Conclusions</u>: We have examined and used a range of statistical methods to evaluate side-specific breast cancer risk. An excess of breast cancers occurred in the same breast within the first years of follow-up, especially in women with atypia. This suggests that precursors may exist within the spectrum of benign breast disease that can be identified with molecular techniques and targeted with tailored interventions.

MULTIFOCAL ATYPIA CONFERS INCREASED RISK OF BREAST CANCER

AC Degnim[1], D Visscher[1], MH Frost[1], LJ Melton[1], RA Vierkant[1], SD Maloney[1], VS Pankratz[1], TA Sellers[2], WL Lingle[1], T Tlsty[3], H Berman[3], LC Hartmann[1]
[1]Mayo Clinic Cancer Center, [2]Moffitt Cancer Center, [3]University of California San Franscisco
Degnim.Amy@mayo.edu

Background: Atypical hyperplasia is a well-recognized risk factor for breast cancer, conveying a 4-5 fold increased risk. However, risk stratification for women with atypia is clinically desirable yet remains elusive.
Methods: We identified all women with atypical hyperplasia in the Mayo Benign Breast Disease Cohort Study through biopsy specimen review by a single breast pathologist. Histologic details of number of atypical foci and the presence of calcifications were recorded. Standardized incidence ratios (SIRs) of breast cancer were estimated compared to Iowa SEER rates.
Results: Of the Mayo cohort of 9087 women, 336 (3.7%) had atypical hyperplasia. With mean follow-up of 12.2 years, 66 (19.6%) breast cancers occurred. Atypia conveyed an increased risk of breast cancer (SIR 4.4, 95% CI 3.4-5.6). Marked elevations in risk were seen in women with three or more foci of atypia (SIR 9.3, 95% CI 5.8-14.1) and especially for three or more foci with calcifications (SIR 12.8, 95% CI 7.8-19.7). Risk was higher in women diagnosed with atypical hyperplasia before the age of 45 (SIR 7.4, 95% CI 3.6-13.7) versus atypia diagnosed at age 45-55 (SIR 5.5, 95% CI 3.6-8) or greater than 55 (SIR 3.4, 95% CI 2.3-4.9). Risk was similar for ductal and lobular types of atypia, and family history did not significantly increase risk. Breast cancer risk in women with atypia remained elevated over 20 years.
Conclusions: In the presence of atypical hyperplasia, very high risk patients (>50% risk at 20 years) may be identified based on the number of foci of atypia and the histologic presence of calcifications. Family history conferred only modest additional risk in this study.

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Increased Breast Cancer Risk in Women with Papillary Lesions of the Breast

[1]Jason T. Lewis, MD, [2]Lynn C. Hartmann, MD, [3]Robert A. Vierkant, MAS, [3]Shaun D. Maloney, BA, [3]V. Shane Pankratz, PhD, [2]Teresa M. Allers, [2]Marlene H. Frost, PhD, and [1]Daniel W. Visscher, MD

Breast papillomas may be single or multiple and associated with atypical ductal or lobular hyperplasias (ADH/ALH). The risk of breast carcinoma development in patients with papillomas, particularly with multiple or atypical lesions, is incompletely defined. Fibrocystic lesions were histopathologically classified in a benign breast disease cohort of 9155 who underwent biopsy from 1967-1991, with papilloma assessment in 9108 of these. Individuals with papillomas (N=480) were classified into four groups: single papilloma (SP, N=372), single papilloma with ADH or ALH (SP+A, N=54), multiple (>5) papillomas (MP, N=41), and multiple papillomas with ADH or ALH (MP+A, N=13). Those without papillomas were classified as non-proliferative (NPFC, N=6053), proliferative without atypia (PFC, N=2308), and atypical hyperplasia (AH, N=267). The relative risk of cancer development within our cohort was compared to that expected in the general population using standardized incidence ratios (SIRs). The relative risk of breast cancer development associated with SP (2.04, 95% CI 1.43-2.81) was greater than NPFC (1.28, 95% CI 1.16-1.42) but similar to PFC (1.90, 95% CI 1.66-2.16). The risk associated with SP+A (5.11, 95% CI 2.64-8.92) was highly elevated but not substantively different than AH (4.17, 95% CI 3.10-5.50). Patients with MP are at increased risk compared to PFC or SP (3.01, 95% CI 1.10-6.55), and particularly those with MP+A (7.01, 95% CI 1.91-17.97). The observed frequency of ipsilateral (vs. contralateral) breast cancer development in papilloma subsets was not significantly different than other patient groups. Single papilloma imparts a cancer risk similar to conventional proliferative fibrocystic change and the presence of atypia in, or associated with, papilloma does not modify the risk connotation of ADH/ALH overall. MP constitutes a proliferative breast disease subset having unique clinical and biologic behavior.